Technical Report No. 32-443

Soil Organic Matter

R. E. Cameron G. B. Blank

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R. E. Cameron G. B. Blank

H. Ford, Acting Chief

Lunar and Planetary Sciences Section

JET PROPULSION LABORATORY

CALIFORNIA INSTITUTE OF TECHNOLOGY

PASADENA, CALIFORNIA

May 23, 1963

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ABSTRACT

A determination of the amount, nature, and distribution of organic matter in desertic soils will be useful for extraterrestrial life detection and the biotic characterization of an extraterrestrial soil environment. Levels of organic matter in southern California desertic soils are given in terms of carbon and nitrogen and the total organic matter content.

A direct determination of soil organic matter by the autoclave colorimetric method is presented for the first time, and it is compared with standard methods for the indirect estimation of soil organic matter from carbon and nitrogen values. The autoclave colorimetric method correlates with indirect methods and could secondarily provide information on soil texture.

I. INTRODUCTION

A determination of soil organic matter and its constituents is of vital importance in any program for detection and characterization of extraterrestrial life. It is especially significant in a life-detection program since it is itself of biotic origin, its nature and composition are dependent upon the kinds of organisms from which it is derived, it is partially composed of animate matter (living organisms), and it is a biotic process-its decomposition and cyclic transformations are an essential feature of life. It is also significant that it is a universal soil characteristic on this planet. There are no terrestrial soils which do not contain at least some organic substances, whether of exogenous or endogenous origin, but the amount may be minute. In harsh, desert environments, for example, the total amount of organic matter present in the soil is comparable to the amounts found in igneous rocks, limestones, and shales (Ref. 1). The soil organic matter in such relatively barren areas need not be of macro plant or animal origin, and can be entirely microbial in nature. This is an important consideration in current life-detection experiments since they are primarily designed to discern the presence of soil microorganisms, their activities, or products.

Of the limiting soil factors necessary for existence of its indigenous biota, the organic matter content is one of primary importance. The nonliving organic materials in the soil are, in general, the food and/or energy source for the living fraction of the soil ecosystem—the populations and communities of microorganisms, as well as macro plants and animals, which are dependent upon the soil for their sustenance. In this respect, the kinds of microorganisms, their abundance, and their distribution are largely determined by the amounts and kinds of organic matter present. A large community of soil organisms is found in soils relatively high in organic matter content, and soils low in organic matter have small numbers of organisms regardless of the inherent and eventual decomposed state of the organic matter. A high number of zymogenous taxa, particularly the saprophytes, are found when organic matter is abundant, and a comparatively greater proportion of the autochthonous flora, including the autotrophs, are found in less favorable and correspondingly less dynamic soil ecosystems.

Activities of microorganisms are also regulated to a great extent by the soil organic matter content. Following the introduction of a suitable organic material into the soil, its decomposition is dependent upon the nature and kinds of organisms present, climatic influences, and edaphic factors of the microenvironment. Therefore, when organic matter is introduced into a terrestrial-soil ecosystem, the numbers and activities of certain microorganisms will increase at the appropriate stage in its degradation. However, increase in abundance and activity of any particular group of microorganisms is especially dependent upon the nature of the organic matter, its stage of decomposition, and upon favorable conditions of the immediate soil microenvironment, particularly moisture and temperature. It may for this reason be necessary to anticipate the minimal soil environmental conditions, as well as nutritional requirements, necessary for the detection of any indigenous extraterrestrial soil microorganisms.

There is at present no reason to assume that any extant, endemic extraterrestrial microbiota would utilize a particular terrestrial source of organic matter or one of its specific constituents. Although a number of soil organisms show nutritional specificity (glucose, for example, is readily decomposed by many soil microorganisms), it cannot be concluded that such a substance would be utilized in an extraterrestrial habitat or under extraterrestrial environmental conditions. In like manner, it cannot be concluded that the distribution of an extraterrestrial microflora will be similar to that on Earth. But, the possibility also exists that in terrestrial environmental conditions which may resemble an extraterrestrial environment, particularly in unique terrestrial microecological habitats at extreme limits of the natural environment, that a similar endemic extraterrestrial community or population of soil microorganisms could exist, metabolize, and reproduce. However, this could require the development of detection techniques for particular, unique groups of microorganisms in specific microenvironments. Regardless of the environmental situation, in terrestrial soils it is generally shown that the distribution and abundance of microorganisms is strongly correlated with the abundance and distribution of soil organic matter. The detection and determination of soil organic matter would therefore most likely include many widelydistributed living microorganisms as well as inanimate organic substances.

A general determination of soil organic matter is also of notable value in that a processed sample is not necessary; it does not require a separation of sorbed organics from the mineral constituents of the soil, and it does not require specificity for a particular organic component. For detection purposes, a processed sample may even be detrimental if a comparatively large fragment of organic material is discarded by means of a size-limiting process. And, in regard to dispersion of soil organic matter, it may be extremely difficult to separate tenacious microorganisms as well as sorbed nonliving colloidal organic matter from soil particles (Ref. 2). In terrestrial soils, a number of microorganisms can be found within this colloidal film surrounding soil particles, and it may therefore be disadvantageous to attempt separation of the microbiota from soil particles if there is the possibility that the adherent microorganisms will be discarded along with the soil particles. Furthermore, there is a strong indication that calcium, which is sometimes abundant in desert soils, may be a chief interfering ion in soil organic matter extraction (Ref. 2). Finally, the value of a total organic matter determination rests upon nonspecificity, except for its major elemental constituents of organic carbon and nitrogen. It does not require the detection of unique compounds or substances such as biotin, DNAase, glycine, etc., which are present in microgram quantities per gram or 100 g of productive soil. Such substances, although they are important constituents of terrestrial organic matter, may not be present in an extraterrestrial soil, or, if present, probably in such micro amounts as to be undetectable without resorting to refined techniques or processing of a large quantity of soil materials.

In regard to soil organic matter as a whole, it can be emphasized that an organic-matter analysis in terrestrial soils is based upon the determination of an accumulative admixture of all organic materials and substances of varying amounts, physical subdivision, composition, and biotic state. Primarily, it is a determination of the organic carbon and nitrogen—both basic, essential elements of terrestrial life, which are complexed within the framework of the total soil organic matter. In an appropriate determination of soil organic matter one must therefore distinguish between forms of organic carbon and nitrogen which are of biotic origin; e.g., protein, humic acid, or fulvic acid, and not of an inorganic nature; e.g., carbonate or nitrate. However, even these latter substances are of importance since they, too, are the result of biotic

influence and frequently represent two of the endproducts of microbial metabolism. It is also significant that water, essential to terrestrial life, must have been present for their formation.

II. OBJECTIVES

It is necessary that background information be obtained on the organic matter content of terrestrial soils at various depths in order to design and instrument life-detection experiments sensibly. It is also necessary that standard methods for the determination of soil organic matter or its major elemental constituents be investigated for their sensitivity and adequacy. Furthermore, it is desirable that new methods be proposed and investigated which might be useful in life-detection experiments and in the biological characterization of extraterrestrial soils. In this regard, colorimetric or turbidimetric methods should be considered for their applicability to instrumentation presently under consideration. Finally, it is desirable that any additional information resulting from this study that would influence proposed

life-detection experiments and the investigation of extraterrestrial soils should be presented at this time.

The purpose of this research is fourfold: (1) to determine the levels of organic matter present in desertic soils that are representative of harsh terrestrial environments low in organic matter as well as moisture content, (2) to determine the amounts of organic matter present in these soils by standard methods, (3) to determine the value of a new colorimetric method devised for the direct determination of soil organic matter, and (4) to provide supplementary information on other soil properties, e.g., soil texture, which might be of value in the investigation and characterization of extraterrestrial soils.

III. METHODOLOGY

Determination of the organic matter content of soils can be divided into three categories: (1) direct: a determination of the total organic material in the soil, (2) indirect: a determination of the major elemental constituents of soil organic matter which can serve as a basis for estimating total soil organic matter, and (3) proximate analyses: determinations of specific extractable, hydrolyzable, and thermolabile organic constituents of the soil.

Total organic matter is most frequently determined in soils by direct and indirect means. Direct methods include: (1) ignition (combustion or pyrolysis) at elevated temperatures, (2) acid digestion; e.g., with perchloric acid, or hydrochloric-hydrofluoric acid, and (3) oxidation with hydrogen peroxide. Usually these are gravimetric procedures whereby the loss in weight of a soil sample is assumed to be the total organic matter content. Indirect methods of analysis involve the determination of

oxidizable organic carbon or nitrogen by wet or dry combustion procedures using acids and/or heat, and the subsequent release of the organically combined carbon or nitrogen. After treatment with strong acids, carbon is commonly determined through back-titration with a reducing substance or determined gravimetrically as sorbed carbon dioxide. Nitrogen is commonly determined titrametrically after the nitrogen in the soil has been subjected to digestion in acid at high heat and converted to ammonia, or determined gravimetrically after combustion and sorption as ammonia or molecular nitrogen. No matter what method is used, precautions must be taken to preclude or compensate for inorganic forms of carbon or nitrogen before or after proceeding with determinations of the oxidizable organic forms. Variations are used in the above methods which relate to the measurement of carbon or nitrogen by colorimetric, turbidimetric (nephelometric) or electrometric (conductimetric) means.

Proximate analyses of the soil organic matter are more complex than the above, and refer to the extraction, isolation, purification, or characterization of more complex fractions of the soil organic matter, such as the fulvic acid complex, protein, polysaccharides, and fats and/or more specific components within each group, e.g., phenolic glucosides, glycine, cellulose, or oleic acid. Analyses for these fractions relate to objectives that are more concerned with the specific chemical nature of a soil organic fraction and have not been considered as indicative of the total soil organic matter present.

All of the above procedures have merits as well as certain disadvantages and their value is limited not only by the specific method used, but upon the interpretation given to it. In many cases difficulties are encountered in analyses due to the nature and character of interrelated soil properties, e.g., pH, Eh, buffering capacity, salt concentration and composition, texture, structure, and mineralogical composition. Consequently, it is highly advisable, and oftentimes necessary to have supplementary information on the nature of certain soil properties which may influence or adversely interfere with the determination of a particular soil constituent.

IV. MATERIALS AND METHODS

The experimental materials consisted of 34 virgin, California soils collected from the Colorado, Mohave, and Great Basin Deserts from the soil surface to depths of three or four feet. Twenty-seven of these soils from the Colorado and Mohave deserts were characterized previously according to pertinent physical attributes (Ref. 3). One additional soil, No. 60, from the Mohave Desert and six other soils, No. 3, 4a, 5, 6a, 7, and 8, from Owens Valley in the Great Basin Desert have since been procured. All of these soils were carefully collected to exclude contamination, passed through a 10-mesh screen (sieve openings of 2.0 mm), and thoroughly mixed. In some cases, particularly where necessary to limit the sample size for analysis purposes, the soil material was ground to a fine powder in order to assure a more homogenous sample and provide for a more favorable reactivity. For dry combustion procedures, and when necessary to use a sample of 200 mg or less, the soil was pre-dried to a condition of pF 7 (oven-dry state of 105±5°C for at least 12 hr to remove moisture). Otherwise, air-dry soils were used which were equilibrated to approximately 50% relative humidity.

The methods used for determination of soil organic matter included (1) estimation of total organic matter by ignition in a muffle furnace, (2) determination of carbon by (a) wet combustion using the Walkley-Black procedure (Ref. 4), (b) wet combustion according to the procedure of Allison (Ref. 5), and (c) dry combustion with the Coleman Carbon-Hydrogen Analyzer (Ref. 6), (3) determination of nitrogen by (a) Kjeldahl method (Ref. 7), (b) dry combustion with the Coleman Nitrogen Analyzer (Ref. 8), and (4) colorimetric determination of the total soil organic matter after hydrolysis by autoclaving. The first three methods are "standard" for analysis of soil organic carbon and nitrogen. The colorimetric method used here is presented as a new procedure and involves methods and principles not employed previously in determinations of soil organic matter. All of the above methods are given briefly as follows:

(1) Ignition Method: A gravimetric method whereby a tared soil sample is subjected to 700°C in a muffle furnace for 30 min, cooled, and reweighed. The difference in weight, which may be compensated for loss of carbonates, is given as the percent of total organic matter in the soil.

- (2) Walkley-Black Method: A wet combustion procedure whereby a known volume and concentration of chromic acid is used to oxidize a soil during spontaneous heating following dilution with concentrated sulfuric acid. The solution is back-titrated with a reducing substance, usually ferrous ammonium sulfate or ferrous sulfate, in order to determine the exact amount of chromic acid used to oxidize the organic carbon. This determination of soil organic matter necessitates the use of a conversion factor, and variables in the method include consideration of concentrations and proportions of chromic acid and sulfuric acid, time of reaction, heat of reaction, compensation for interfering ions and care to be sure that over one-half of the chromic acid is not consumed in oxidation of the organic matter (Ref 9).
- (3) Allison Method: A second wet combustion method also involving chromic acid, sulfuric acid, and external heat. Carbon dioxide is evolved through a carrier stream passed successively through precautionary traps containing potassium iodide, silver sulfate, sulfuric acid, zinc, and anhydrone, after which the gas is sorbed on Mikohbite and weighed. Pretreatment is customary for removal of carbonates in an open flask before continuing the procedure for organic carbon. Sufficient chromic acid must be utilized and the traps must be capable of sorbing interfering substances from a predetermined adequate weight of soil.
- (4) Coleman Carbon-Hydrogen Analyzer, Model 33 Method: A dry combustion method whereby a weighed sample of 50 to 200 mg is placed within a pyrolysis tube containing pertinent inorganic substances, and subjected to an atmosphere of oxygen with a temperature of approximately 850°C. Subsequently, the gas is passed through the carrier stream and the evolved carbon dioxide is sorbed and weighed. This apparatus must have mechanical, chemical and electrical maintenance. For example, the system must be checked for leaks, the combustion time and temperature controlled, and the chemical constituents of the pyrolysis tube checked and renewed. The sample size and its state of subdivision must be carefully regulated, and hysteresis effects have been noted which are dependent upon the carbon content of successive samples.
- (5) Kjeldahl Method: A wet digestion method for combined organic nitrogen and ammonia which utilizes

concentrated sulfuric acid and an inorganic digestion mixture which also regulates the temperature of the reaction. After a sufficient time interval, all of the organic nitrogen in the sample is converted to ammonia. The mixture is next neutralized with strong base, the ammonia distilled over (usually into a boric acid solution), and the amount of nitrogen determined titrametrically, commonly with dilute hydrochloric acid. Digestion time and temperature are crucial and neutralization must be performed with care. The sample size, as for other methods, is dependent upon the amount of organic material (including ammonia) present in the soil.

- (6) Coleman Nitrogen Analyzer, Model 29 Method: The procedure and apparatus are somewhat similar to that for the Coleman Carbon-Hydrogen Analyzer. Some major exceptions are that the carrier stream is carbon dioxide, the nitrogen gas is scrubbed in a caustic solution and its volume is collected and measured in a microliter chamber attached to a digital counter. Pyrolysis tubes of a larger diameter were constructed for use with soils and the recommended maximum sample size of 50 mg increased to 0.5 to 1.0 g.
- (7) Colorimetric Method: This method is based upon the development and intensity of colored hydrolyzable organic products obtained by means of an autoclave. The soil extract is subsequently referred to known color standards. The procedure is as follows: (a) equal proportions of soil (50 g soil: 50 ml water) are mixed in a loosely stoppered Erlenmeyer flask, and a few additional mls of water are added to compensate for water lost during the heating cycle. The soil-water mixtures are subsequently autoclaved for 30 min at 250°F and 15 psi pressure. After the solutions have cooled to approximately room temperature, the extract is decanted into a colorimeter tube, or centrifuged if necessary to hasten sedimentation of colloidal soil particles, and the optical density determined in a Klett-Summerson photoelectric colorimeter using a blue filter of 400-425 m_{\mu}. The optical density values for the soil extracts are then referred to a curve, Fig. 1 (Curve A), which was determined from

known concentrations of powdered yeast extract¹ used for the organic matter reference standards. The values obtained for the soil extract are considered as percent total soil organic matter. It assumes that concentrations, optical densities and color obtained for hydrolyzable, microbial products, i.e., yeast extract, can be utilized for estimating total soil organic matter when soil extract is obtained by hydrolysis in the autoclave, and its resulting optical density and color plotted against the standard curves.

Other values, Table 1, were obtained for the soils as follows: (1) mechanical analysis for grain size distribution by the hydrometer method (Ref. 10), (2) textural class by reference to a soil textural triangle (Ref. 11), (3) color of air-dry soil by comparison with standard soil color charts (Ref. 12), (4) iron and manganese by spectrographic analysis of finely ground material (Ref. 13), (5) inorganic carbon by acid digestion and gravimetric determination of the carbon dioxide evolved (Ref. 5), and (6) nitrate nitrogen by the phenoldisulfonic acid method (Ref. 14).

¹Procured from Baltimore Biological Laboratory, Baltimore, Md.

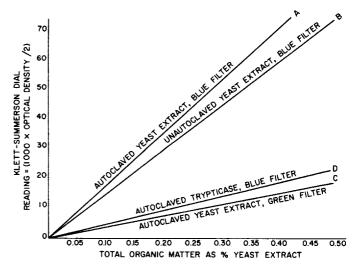


Fig. 1. Standard curves for colorimetric measurements of soil organic matter

Table 1. Supplementary information on soils

														_						_				_		_	_	_	-	_		_	_		_	_	_	_			
32	Org. C	system)	0.25	0.07	0.07	0.07	0.05	60:0		0.27	0.70	0.24	0.24	14.0	0.22	0.12	0.10	0.15	0.26		0.10	0.12	600	0.00	2 5	7		0.62	0.55	0.22	0.22	0.18	0.14	:	0.13	- 6	0.0	90.0	0.12	0.13	0.17
3	W-B. Org. C	(no heat)	0.24	0.04	0.05	0.02	0.05	0.04		0.17	0.0	0.09	0.00	2	0.17	0.10	60'0	90.0	60.0		80.0	90.0	0.07	0.08	0.02))		0.53	0.39	0.14	0.07	0.05	0.02		0.14	0.08	0.0	0.04	90.0	0.08	0.11
30	Mean Org. C/	Z lefe Z	7.1	8.8	8.8	8.3	10.0	6.4		2.8	\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \	- ·	0.4	2	70	12.2		17.1	10.9		0.9	9.5	6.3	5.5	ç; ç	7.4		6.9	0.6	9.5	10.0	15.0	18.0	Ş	10.6	9:0	0.0	4.2	6.9	6.5	8.5
23	Medin Org. C/	Kjeldahi N	11.5	14.0	35.0	50.0	25.0	35.0		9.6	5.5	7.3	13.8	0.0	8	183	25.0	24.0	24.0		10.9	10.9	10.0	0.:	0.1.0	5.5		14.7	8.7	6	14.5	17.1	22.5	•	15.5	13.3	 	16.0	18.3	18.3	17.1
58	N % ESW	Š	< 0.02	< 0.02	0.41	0.30	90.0	1.40		0.59	1.30	0.39	1.20	67.4	8	0.54	0.31	2.10	2.00		0.13	0.23	0.04	0.06	0.06	2		0.20	0.71	0.25	0.27	90.0	0.45	:	0.50	0.30	0.60	0.46	0.48	0.40	0.65
27	e i	-+	0.15	0.14	0.12	0.13	0.11	0.07		1.00	86.0	0.91	0.93	7.74	0.74	790	0.00	0,60	1.0.1		00.0	0.00	0.00	< 0.01	1.0	 		41.0	0.03	0.03	0.16	0.19	0.11		0.01	00:0	0.00	0.00	0.02	90.0	0:30
76	% Total	nogue	0.44	0.24	0.23	0.22	0.21	0.22		1.27	1.30	71.1	1.21	^	771	1 8		0.80	1.27		0.14	0.16	0.11	0.20	0.27	0.27		, a	9.0	0.27	0.45	0.50	0.29		0.22	91.0	0.13	0.13	0.17	0.19	0.53
22	ssol %	ignition	2.11	1.59	1.32	1.08	99.0	1.41		8.29	7.71	7.65	6.94	14./	4.43	23.3	40.0	100	4.80		0.98	1.20	1.13	2.96	3.04	3.34		200	2 44	25.	1.75	2.42	1.82		71.1	1.30	=	1.05	1.28	1.78	2.93
7.	% Wu		0.056	0.048	0.042	0.028	0.035	0.041		0.083	0.062	0.047	0.069	0.047	3000	0.080	0.040	0.025	0.062		0.047	0.036	0.040	0.066	0.055	0.042		1500	8000	0.035	0.036	0.034	0.031		0.057	0.037	0.053	0.083	0.056	0.047	0.050
23	% F•		17	1.5	2.0	0.78	1.7	1.9		2.7	2.7	2.7	2.7	7.0	7	5.0	5.7	. 7 6	<u>.</u>		2.0	1.8	8.1	2.7	5.6	2.4		-		4 4	2 2	7	Ξ		=	<u>-</u>	5.7		<u>.</u>	9.	1.85
22	Munsell soil	color (dry)*	light aray	light aray	light gray	liaht aray	light gray	light gray		light reddish brown	light reddish brown	light reddish brown	light reddish brown	light reddish brown		light olive gray	light olive gray	light office gray	light olive gray		pale brown	pale brown	pale brown	yellowish red	yellowish red	yellowish red		111111	pinkish gray	E-Le vallawish branch	light vellowish brown	light yellowish brown	very pale brown		pale brown	pale brown	very pale brown	light aray	pale brown	pale brown	*Sieved sample
21	Textural	class	paos	sand	Sand	sand	puas	sand		day	clay	clay	clay	clay		sandy loam	mpol ypubs	sandy loan	sandy foam		sand	sand	sand	sandy loam	sandy loam	sandy clay	mpol	-	puos	Pup.	pubs	sand	puos		sandy loam	sandy loam	sandy clay	sandy loam	sandy loam	sandy loam	
	sis	% Clay	20	0.4	7.4	3.6	0.4	3.7		45.8	48.8	45.8	47.4	47.6		4.0.7	0 c	4 0	14.6		4.1	5.5	4.6	19.6	15.7	24.9			4.4	5.0	. 0	8.1	6.4		6.7	10.7	21.6	14.6	14.7	17.8	
90	Mechanical analysis	₩15 %	4.5		2.0	2.8	80	4.9		18.0	15.6	16.6	14.6	15.8		8, 12	0.12	0.6	23.8		4.5	3.1	8.8	6.3	7.0	6.4			5.3	0 6	7.0	2.0	5.5		12.7	17.8	9.9	17.3	17.8	18.0	
	Mech	% Sand	01.7	000	63.3	93.6	87.2	4.19		36.2	35.6	37.6	38.0	36.6		9.1.8	97.9	07.0	5.70		91.4	91.4	86.6	74.1	77.3	68.7			2.00	4 1	07.7	84.0	88.1		77.6	71.5	61.8	78	57.5	64.2	
_	Depth	•	surface to	1/16 15.	1/10 \$5 - 11.		. 4	3 +	surface to	1/2 in.	1/2 to 6 in.	#-	2 #	3#	surface to	1/8 in.	1/8 to 6 in.	# 4	3 4	curfore to	1/4 in.	1/4 to 6 in.	. =	2#	2-1/2 ₦	3#		surface to		1/4 to 0 in.	± 4 - c	= 45	. †	surface to	ë	1 to 6 in.	# -	4	= #	: +	All Depths
	Soil	ź					_	5.45		9	89	69	63	5		₹ :	62	% :	4 7	+	74	2	_	72	73	%			7.2	۰ ۱ ۲	\		: 16		6	40	٠,	7	8 1	. œ	West

V. RESULTS AND DISCUSSION

A. Organic Matter in Desertic Soils

Average values for organic carbon and organic matter by various methods for the 34 soils are given in Table 2. Columns 1, 2, and 3 are results for direct determinations of organic carbon with compensation or pretreatment to exclude inorganic (carbonate) carbon. Carbon values in columns 4, 5, and 6 were indirectly determined as indicated. The figures in column 4 were calculated after determination of the organic carbon in soil extracts subsequent to the autoclave procedure outlined above. A factor of 10 was applied because approximately one-tenth of the total organic carbon can be removed from soil by hot water extraction.2 Organic carbon can also be computed from the nitrogen value obtained by the Kjeldahl method when multiplied by a factor of 11.7 as shown in column 5. This latter factor has been derived from soils by assuming a 5% nitrogen content and a C:N ratio of 11.7 to 1 (Ref. 15). In column 6 the organic carbon has been calculated from organic matter values by means of the conventional factor of 1.724. This factor is commonly used to estimate total organic matter in soils subsequent to the direct analysis for organic carbon; it is based on the assumption that soil organic matter is 58% carbon (a ratio of 1-to-7 between organic carbon and soil humus). This same factor has been used to calculate the soil organic matter content indicated in columns 11, 12, and 13. The direct determination of total organic matter by the colorimetric method provided values shown in column 15. An estimation of soil organic matter has also been calculated from the Kjeldahl nitrogen values, column 9, by applying the conversion factor of nitrogen \times 20, as shown in column 14. Kjeldahl nitrogen (organic or amino forms) is usually a fraction of the total nitrogen, column 10. Indirect calculation of organic matter from the Walkley-Black determination of organic carbon in soil extracts is given in column 16. An additional factor of 27.90 is applied since this percentage of organic carbon could be recovered by means of the same procedure for known concentrations of aqueous solutions of yeast extract.

Average amounts of carbon for any one soil as determined directly are reported in column 7; average amounts of carbon which were derived indirectly through appli-

cation of conversion factors are shown in column 8. Averages for organic matter in any one soil as determined indirectly are given in column 17, and followed by variability in observations as indicated by the standard deviation and standard error in columns 18 and 19, respectively. Mean values for all depths of soil and all collection sites are given at the bottom of each column and can serve for generalized comparison purposes in levels and amounts of organic matter.

The correlation of five methods used for determining the amount of soil organic matter are given in Table 2. The Allison method shows the strongest correlation, 92% or more, with the Walkley-Black, Kjeldahl and colorimetric methods. It should show the strongest correlation with the Walkley-Black method, which it does, since these two are essentially the same combustion procedure. Other strong positive correlations are also indicated in Table 3, with percentages of 85% or above. The Carbon-Hydrogen Analyzer shows less correlation with other methods, particularly with the Kieldahl method. A greater average percent of organic matter, 0.40%, is indicated by means of the Carbon-Hydrogen Analyzer as compared with the Kjeldahl method with 0.28%, but this latter amount is comparable with the average amount obtained by the Allison, Walkley-Black and colorimetric methods. The colorimetric method shows a good to strong correlation of 74.8, 92.0 and 89.2% with the three methods used for estimating the total percent organic matter in the soil by means of conversion factors. This also supports the validity of the conversion factors, which is remarkable when it can be considered that the use of these factors is somewhat dubious for a random soil sample, and a specific factor should be determined analytically for each soil. It has been shown, for example, that factors for conversion of carbon to organic matter can range from 1.6 to 3.3 (Ref. 16). The indirect estimation of organic matter from soil nitrogen values is likewise quite questionable (Ref. 17), especially for a single sample of soil, but it can give a comparatively accurate figure for a group of soils (Ref. 15).

The range of soil carbon, nitrogen, and organic matter for any one soil depended to some extent upon the method used. For direct determination of organic carbon, these ranges were as follows: (1) Carbon-Hydrogen Analyzer, 0.10–0.70%, (2) Allison, 0.06–0.62%, (3) Walkley-

²Calculated from values obtained by Vandecaveye and Katznelson (Soil Science, Vol. 46, p. 152, 1938); the mean value which we obtained for their 24 soils was 12.14, with a range of 7.71 to 17.33.

Table 2. Desertic soil organic carbon, nitrogen and organic matter values

	2	S.E.	03	10.0	02	03	05	3	_	0.02	9	50	03	60		-	0.03	05	5	0.5		10	6	- 10	 5	0.02	70		2 5	3 6	50	8	70.0		50.	.02	.03	.02	0.03	.03	0.04
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	=	S.D.	90.0	0.03	0.05	0.06	0.05	60.0		0.04	0.13	0.12	90.0	0.21		0.38	0.07	0.1	0.03	0.1		0.02	0.03	0.03	0.03	0.05	0.0			5 6	210		0.10		0.12	0.04	90.0	0.05	0.07	80.0	0.0
	- 11	Mean (Columns 11-15)	0.45	0.12	0.11	90.0	60'0	0.12		0.47	0.40	0.36	0.42	0.68		0.54	0.18	0.17	0,21	0.21		0.21	0.23	0.16	0.20	0.21	0.17		- 6	0.50	0.50	200	0.14		0.30	0.21	0.16	0.15	0.20	0.16	0.30
	16	W-B extract C ×10×27.90	1.28	0.20	0.22	41.0	0.11	90.0		0.36	65.0	0.27	0.33	68.0		1.09	0.27	0.22	0.25	0.25		0.33	0.33	0.17	0.33	0.27	0.20	3	9	0.73	0.00	35.0	0.22		0.33	0.20	0.17	61.0	0.17	0.20	0.39
% Organic matter	13	Celerimeter direct O.M.	0.38	0.10	60.0	0.04	90.0	0.07		0.50	0.42	0.33	0.42	0.85		0.37	0.13	60.0	91.0	41.0		0.24	0.20	0.13	♦1.0	0.17	0.20		6.	0.80	0.28	7 0	0.07		0.48	0.21	0.24	0.18	0.27	0.27	0.29
%	*	Kjeldahi N×20	15.0	0.10	0.05	0.02	0.03	0.05		0.51	0.49	0.51	0.50	0.92	-	0.47	0.12	60.0	0.10	0.10		0.23	12.0	61.0	0.20	0.21	0.12		0.92	61.1	5.0	77.0	0.07		0.21	0.19	60.0	0.10	0.12	0.11	0.28
	13	W-B C×1,724	0,40	0.12	60.0	20.0	60.0	60.0		0.40	0.24	0.24	0.31	0.53		0.29	0.17	0.15	0.22	0.15		₹1.0	0.22	0.12	≯ 1.0	0.17	0.15		16:0	0.78	0.33	0.70	100		0.22	0.10	0.12	0.12	0.15	91.0	0.24
	13	Alitson C×1,724 C	0.43	0.13	0.12	0,11	0.11	0.15		0.47	0.28	0.26	0.41	99.0		0.38	0.20	0.18	0.25	0.20		91.0	0.25	91.0	91.0	0.21	0.21		1.07	76.0	0.37	0.78	• • •		0.23	0.20	0.15	71.0	0.20	0.22	800
		C-H Org. C × 1.724	0.50	0.17	0.19	0,16	0.17	0.26		0.47	0.55	0.45	0.48	0.43		1.21	0.29	0.36	0.34	0.45		0.24	0.28	61.0	0.34	0.28	0.17		•	1.05	0.41	0.30	5.0	+	7,0	0.28	0.22	0.22	0.26	0.22	0.40
	10	Total ×			80	- %	000	110.0	-		0.047	131	335	184		335	600	600	200	0.011		250	0.013	910	020	0.020	910	-	170	928	120	•	0.008		710	7	810			0.017	0000
% Nifregen	Ĺ		-	- 0	9.0	9.0	0.0	7.0	_	9.0	7.0	9.0	9.0	7.0			-											_				_	_	+				-			+
8	٥	Kjeldohi R	0.026	0.005	0.002	0.001	0.002	0.002		0.026	0.024	0.026	0.025	0.046		0.024	900.0	0.00	0.005	0.005		0.011	110.0	0.010	0.010	0.010	0.006		0.045	0.060	0.022	10.0	0000		-	200	9000	2000	7000	0.00	100
	-	(Celomns 4-6)	120	0.0	0.05	0.03	0.03	0.03		0.24	0.33	0.20	0.22	0.45		0.29	0.08	90:0	0.08	0.08		0.13	0.12	60.0	0.11	0.11	60:0		12.0	0.49	0.18	0.12	0:0		9.0	9 0	200	70.0	0.0	0.10	1.0
	,	Direct mean (Colomns 1-3)	96.0	0.08	0.08	0.07	20.0	0.10		0.26	0.21	0.18	0.23	0.32		0.36	0.13	0.13	0.16	91.0		0.11	0.14	60.0	0.12	0.13	0.10		19:0	0.54	0.22	0.20	9.10	;	4	2.0	200	000	6.00	0.12	9.5
	•	Colorimeter O.M./1.724	0 33	90.0	0.05	0.02	0.03	90.0		0.29	0.24	0.19	0.24	67.0		0.21	80.0	0.05	60.0	90.0		9 1.0	0.12	80.0	80'0	0.10	0.12		0.95	0.49	0.16	0.13	0.05		900	0.10			2 4	91.0	710
rben	•	Kjeldahi N×11.7	92	900	0.02	0.01	0.02	0.02		0.30	0.28	0.30	0.29	0.54		0.28	0.07	0.05	90.0	90:0		0.13	0.13	0.12	0.12	0.12	20.0		0.52	0.70	0.26	0.13	80.0	3	61.0	7 7		900	20.0	90.0	41.0
% Organic carbon	4	W-8 extract C×10	¥.	200	90'0	0.05	0.04	0.03		0.13	₽1.0	0.10	0.12	0.32		0.30	0.10	0.08	60.0	60'0		0.12	0.12	90.0	0.12	0.10	20.0		99.0	0.27	0.12	0.10	60.0	2000	-:	200	200	200		0.07	7.0
	-	Walkley- Black				_	_	0.05	-					0.31						60.0		80.	113	200	80.0	01.0	60.0		7.53	3.45	91.0	51.5	80.0			2.5	200	.00	90.0	0.1	1
	-						_																									_		+							╁
	2	Allison	_	700	0.07	0.07	900	0.09		0.27	91.0	0.15	0.24	0.39		0 2 2	0.12	010	0.15	0.12		0.10	0.14	90.0	0.09	0.12	0.1		.6.0	0.52	0.2	0.14	80.0	}	_	5 6	- 6	0 0	5 6	0.13	41.0
	-	Chorp. C.	900	01.0	0.11	60'0	010	0.15		0.27	0.32	0.26	0.28	0.25		0.70	0.17	0.21	0.20	0.26		41.0	0.16	0.11	0.20	91.0	0.10		0.67	0.61	0.24	0.29	0.31	5	5	7.0	5.0	5.0	21.0	0.13	
		ŧ.	surface to	5 3 5 3 5	, t			: #	turface to	ž.	1/2 to 6 in.	#	5 th	3.4	1	N in	9 9	#	2 4	: # : #	ri e il	Morrace ro	74 to 6 in.	±	2#	21/2 #	3#	surface to	. <u>£</u>	¼ to 6 in.	=	5 #	± 4		surface to		2 4			: #	4000
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Table 3. Correlation of methods

Methods	% Correlation
1. C-H ¹ , Allison ²	85.0
2. C-H, W-8 ³	81.9
3. C-H, Kjeldahl ⁴	51.3
4. C-H, Color. ⁵	74.8
5. Allison, W-B	95.7
6. Allison, Kjeldahl	92.7
7. Allison, Color.	92.0
8. W-B, Kjeldahl	88.5
9. W-B, Color.	89.2
10. Kjeldahl, Color.	85.2

¹C-H = Carbon-Hydrogen Analyzer ²Allison = Method of F. E. Allison ³W-B = Method of Walkley-Black ⁴Kieldahl = Macro Kieldahl

⁵Color. = Colorimetric method with yeast extracts

Black, 0.04-0.53%. In regard to nitrogen, except in two cases, Kjeldahl values were lower than the total nitrogen figures. Nitrate-nitrogen figures, Table 1, column 28, did not constitute a substantial amount of the total nitrogen, and ranged from less than 0.02 to as much as 4.25 mg per 100 g of soil. Kjeldahl nitrogen ranged from a low of 0.001% to a high of 0.46%. The latter figure was obtained for a 3-ft level of well-drained, desert red clay soil, No. 61, which also had the highest level of nitrate (and salts) than any of the 34 soils examined. Organic matter levels ranged from 0.04 to 1.63%, depending again on the method used, whether direct or indirect, and the conversion factors applied. For the various methods, mean values ranged from 0.08 to 1.14%, usually with the highest amount of organic matter found at the immediate soil surface to a depth of less than an inch.

The highest amount of organic matter was obtained for a sandy soil, No. 75, from the Mohave desert, although it is commonly assumed that, in general, the finer the soil texture the more the soil organic matter and nitrogen is found (Ref. 15 and 18). There are, however, exceptions to this which have been pointed out in earlier work (Ref. 19), showing that fine texture is not associated with high total nitrogen and organic matter content. Some of this organic material was dry organic surface litter, but in other cases it can be attributed to algal and lichen soil crusts which harbor an abundance of various kinds of microorganisms in favorable and unique microecological habitats exposed to direct solar radiation, extreme diurnal fluctuations in temperature, wind, erosion and abrasion, and desiccation. Contrary to what has been reported by Shields (Ref. 20), the rapidity of establishment of the algal component of these habitats is not directly dependent on the presence of fine-textured soil.

Regardless of soil texture, such habitats form noticeable "deposits" of animate and inanimate organic matter in terrestrial deserts, and could possibly be encountered in extraterrestrial situations.

In a number of terrestrial soils, total organic matter has been determined by loss in weight following ignition, as shown in column 25. This method is not applicable to desertic soils, primarily due to the concentrations and kinds of salts encountered; e.g., hydrated magnesium or sodium carbonate. For the 34 soils investigated, the amount of organic matter estimated by this method ranged from 0.66 to 8.29\%, with the highest values reported for the series of clay soils, No. 6 through 61. Part of the weight loss of an ignited soil can be attributed to its salt content, particularly the amount of carbonate (or bicarbonate), column 27, which is sometimes a significant amount of the total carbon, column 26, but this cannot account for the comparatively large loss in weight of an ignited, oven-dry soil. Some of this weight loss could be attributed to "bound" water, although only a few additional tenths of a percent moisture could be obtained by raising the temperature from 100 to 200°C and directly determining the released moisture by exposure to a phosphorus pentoxide electrolytic cell. However, higher temperatures would probably release water of crystallization from the lattice structure of soil particles.

Of particular significance in evaluating the relationship and nature of the carbon and nitrogen status of soils is the determination and examination of carbon: nitrogen ratios. These figures are given in columns 28 and 29. Even though the nitrogen levels in virgin desertic soils are lower than for other soils, it can be seen that there is a significant difference between the ratio of mean organic carbon to Kjeldahl nitrogen and the ratio of mean organic carbon to total nitrogen. The latter values, column 30, are more indicative of ratios expected for desertic soils (Ref. 21). In this respect, up to 50% of the total soil nitrogen may be in the form of protein and about one-third of the organic nitrogen is commonly protein (another organic form of chitin). Part of the soil nitrogen is undoubtedly in various forms associated with the clay lattice, either as ammonia, nitrate or some other inorganic form, but another part is microbial in nature or in a highly decomposed state. This is indicated by narrow C:N ratios, 10:1, or below, found in desertic soils, which is in turn attributed to the over-all climate contributing to arid areas (Ref. 23). The mean organic C:N values for Kjeldahl nitrogen ranged from a high of 50.0 to 1 to a low of 7.3 to 1. The C:N values for total nitrogen were lower and ranged from a high of 18.0 to 1 to a low

of 4.2 to 1. For soils in general, it is commonly stated that the C:N ratios become narrower with the depth of the soil profile. However, this cannot be assumed for desertic soils, and the converse was true even for the ratios of mean organic carbon to Kjeldahl nitrogen. In this regard, for each of the six profiles examined, the widest ratios were obtained at some level below the soil surface!

Of supplementary importance is the relationship between soil organic matter content and the soil color, the latter indicated in column 22. It has been commonly assumed that the darker colored soils are higher in organic matter content, although mineralogical content (and texture) is acknowledged to have some influence in contributing to black, brown, and gray colored soils (Ref. 23). Actually, the amount of organic matter in desertic soils has little influence on soil color. Even when the organic matter content of desertic soils is doubled and the soil heated to darken the added organic material (i.e., starch), no appreciable darkening of the soil could be observed visually or following examination with a reflectivity meter. Of more significance in determining the darker colors of desertic soils are (1) parent material, (2) mineralogical composition and weathering, especially the percents of iron, column 23, and manganese, column 24, and their oxidation state, and (3) soil moisture content, which can cause an appreciable darkening of the soil, although usually not affecting hue, but changing in color value and chroma. Iron and manganese, as well as other mineral elements, frequently form metalloorganic complexes in the soil and these complexes can be a contributing factor to soil color if present in sufficient quantity. The over-all color of desertic soils is greatly dependent upon the nature of the parent material and the amount of weathering that has occurred in that these soils can have occurred over a long time span, but they are commonly young in development and maturity.

B. Colorimetric Method for Soil Organic Matter

The development and measurement of color in soil extracts following autoclaving deserves special consideration in that it correlates well with standard methods used for the indirect estimation of total soil organic matter by means of conversion factors. On this basis, it can therefore be recommended as a suitable method for directly determining total soil organic matter. It differs radically from previous colorimetric methods which depend on the color developed by oxidation of organic carbon with chromic acid. It has been found that the standard colorimetric methods are unreliable in a number of respects,

but primarily in that sample size and digestion reagents must be carefully regulated depending on the expected (or predetermined) organic matter content, carbonate carbon must be precluded, and finally, the color development is not in accord with Beer's Law (Ref. 24). The autoclave method has been used previously as part of the procedure to extract humic acids of coal (Ref. 25), but it has not been used for the quantitative estimation of organic matter in soils.

The autoclave colorimetric method proposed here has a number of merits. Once the standards have been prepared (controls of known yeast extract concentrations may be run with each series of soils if desired), it is rapid, accurate, reproducible, is not affected by "interference" ions such as chloride, or carbonate, requires no grinding of the sample or previous harsh treatment with acids or bases, and it is a direct determination of total soil organic matter without the use of dubious conversion factors. The method is particularly applicable to desertic soils low in organic matter, although as much as 5 to 10% organic matter could also be determined by this method. And, in that it is a colorimetric method, it could have application to presently proposed planetary probes for soil organic matter. It could also give an approximation of soil texture by a method not now in use.

In regard to soil texture, it was noted that the amount of water sorbed by the soil and the amount of swelling of the soil following autoclaving was considerably influenced by soil texture, with the greatest amount of swelling occurring in the clay soils and practically none in the sands. A movable photoelectric beam on a vertical scale would then be able to detect the soil level at the interface of the soil-water mixture, and its height could be noted with reference to known swelling and water sorption of various kinds of soils, and soil-like materials. Salts and organic matter would have some influence on hydration and subsequent volume changes, but this could in turn be monitored by miniature electrodes for pH and conductivity in the soil-water mixture.

As for most methods of analysis, there are certain precautions and disadvantages of the method. For the autoclave colorimetric method, there are at least four important factors in this respect: (1) the autoclave must be in perfect working order for reproduction of time, pressure, and temperature during successive heating cycles, (2) the proper organic matter standards must be used in conjunction with the colorimeter filter of the most adequate wavelength, (3) the influence of oxidation state and solubility of mineral elements such as iron and

manganese must be minimized, and (4) the turbidity of the soil extract must be reduced if necessary. In regard to the four factors mentioned above, the following observations have been made, (1) decrease or increase in the three variables pertaining to the autoclave may decrease or increase the results obtained; the amount of hot-water soluble organic matter may be especially reduced when the pressure and temperature are lowered and the time period shortened, (2) the selection of standards and filters can alter the effectiveness of the method as shown in Fig. 1, and (3) iron and manganese, the two primary constituents of soil minerals which would affect the color of the soil extract, had no discernible effect in their present soil forms on the determination of soil organic matter; however, the influence of iron and manganese organic complexes in proportion to the amount of hotwater soluble organic matter cannot be excluded, and (4) inorganic colloidal matter, particularly the clay fraction can have an appreciable influence, but it was noted that autoclaving definitely promoted the sedimentation of suspended clay even within a few hours for a clay soil, and centrifuging is not necessary except as an expedient to shorten the time of analysis.

Additional comments can be made in regard to the standard methods employed for comparison purposes. The dry combustion method, in general, gave higher results for organic carbon, even though carbonate carbon determined gravimetrically by the Allison method was subtracted from the amount of total carbon. Wet combustion methods, while adequate for most purposes, require much care in preparation of the sample, necessitate consideration of the concentration and proportion of corrosive combustion reagents, the elimination of carbonate-carbon, compensation for interfering ions, e.g., chlorides, active higher oxides of manganese, and ferrous iron (unless the soil has been air-dried for several days), consideration of the stability of the ferrous compound used for back-titration, and regulation of the temperature and reaction time. Some of these considerations were noted previously (Ref. 4). A few examples of the influence of the variables can be pointed out as follows: (1) by means of the Walkley-Black method, the combustion of organic carbon by heat of reaction, column 31, oxidizes less carbon, although it was allowed to react for the same time period using applied external heat, column 3, (2) carbonate carbon influenced to some extent the organic carbon values if the analysis was performed in a closed system, as indicated by figures in column 32, rather than in an open system, column 2, where the evolved carbon dioxide could be allowed to diffuse freely into the atmosphere outside the system, and (3) as shown in Fig. 2, another variable in the amount of organic carbon combusted is the proportion of oxidizing agents, whereby less organic carbon was oxidized with increasing volume and consequent dilution of the reagents.

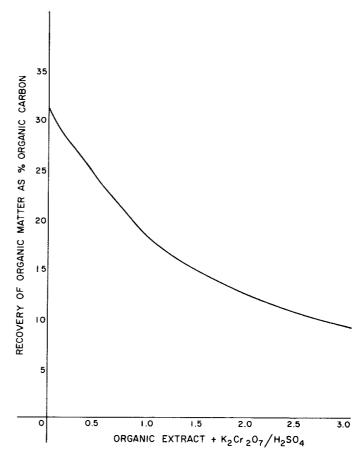


Fig. 2. Recovery of organic carbon as influenced by volume and concentration of reactants

VI. CONCLUSIONS

A determination of total soil organic matter and its constituents is of inestimable value in understanding the nature, activity and development of soils, particularly the attributes of its biological system. A small amount of organic matter, part of which is living matter, outweighs its influence in the soil ecosystem. Any program for life-detection should include some means of not only detecting organic matter *per se*, but insofar as possible, some means for estimating its nature and status.

As indicated by analysis of only 34 soils from three California desert areas, the elemental organic constituents of these soils were quite low, representing the levels found in relatively unweathered materials. The total soil organic matter, whether estimated indirectly or directly is correspondingly low, much more so than for cultivated and/or agricultural soils, particularly in less arid areas (unless fertility is not renewed).

The colorimetric method for determination of soil organic matter by means of the autoclave and with reference to organic extract standards has been shown to be of value as a direct method of estimating total soil organic matter. Whether or not it can be utilized in a space probe in the investigation of extraterrestrial soils, it can still be used as a method for determining organic matter in desertic soils or even less harsh terrestrial environments. Soil texture may be estimated to some extent by the soil-water level following treatment in the autoclave. Wet combustion of organic carbon with chromic acid cannot be recommended for extraterrestrial soil probes at this time without further study. It requires prior knowledge or estimation of the organic matter content of the soil sample, the use of harsh chemicals, and also requires that certain interfering ions either must not be present, must be inactivated, or else precluded.

Regardless of the method used for estimating soil organic matter, it is highly desirous, as well as necessary in many cases, that subsidiary information on soil properties and characteristics be known before reasonable evaluations on soil organic matter can be stated. Such subsidiary information is also valuable for understanding, evaluating, and predicting development, use, activity, and response of planetary soil ecosystems.

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